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# Gravity research on plants: use of single-cell experimental models

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Future space missions and implementation of permanent bases on Moon and Mars will greatly depend on the availability of ambient air and sustainable food supply. Therefore, understanding the effects of altered gravity conditions on plant metabolism and growth is vital for space missions and extra-terrestrial human existence. In this mini-review we summarize how plant cells are thought to perceive changes in magnitude and orientation of the gravity vector. The particular advantages of several single-celled model systems for gravity research are explored and an overview over recent advancements and potential use of these systems is provided.

**Keywords: gravity, gravitropism, gravimorphogenesis, hyper-gravity, micro-gravity, pressure model, statolith, statocyte**

## INTRODUCTION

Long term space missions will greatly depend on the availability of ambient air, sustainable food supply, and treatment of human waste, all of which can be enhanced and improved through the cultivation of plants on-board the space craft (Musgrave, 2007; Wheeler, 2010). Plants also provide calming effects and emotional benefits that can be pivotal in the confined environment of a space craft or orbital platform as they help astronauts to fight loneliness and depression. The positive psychological effects on the crew have the potential to reduce stress resulting from the living and working conditions during a mission (Williams, 2002; Zimmermann, 2003). Because of their multiple roles, plants will play a primordial role in future space missions and understanding the plant metabolic and morphogenetic responses to altered gravity conditions is indispensable for the development of space craft ecosystems or long term planetary colonization at the fractional gravity levels found on the Moon (1/6 Earth's *g*) or Mars (3/8 Earth's *g*).

Cultivation of plants on orbital platforms affects growth of organs and individual cells as was shown in many plant species (Cowles et al., 1984; Kuang et al., 1996; Wolverton and Kiss, 2009; Matsumoto et al., 2010) although in many of these experiments the observed phenomena were a result of the combination of the direct effect of the absence of gravity on the plant and of other environmental factors such as increased radiation or absence of convection. Unlike most biotic and abiotic types of stress which plants have been exposed to during their evolution, gravity is the only constant factor, both in direction and magnitude, to which plants had to adapt in a permanent manner. To withstand the mechanical load imposed by gravity on terrestrial organisms, plants developed mainly two strategies. The first is based on the generation of a hydroskeleton which creates an erectile force based on the balance between the internal turgor pressure and the mechanical constraint by a highly tensile resistant extracellular matrix, the cell wall. The second is based on the fortification of the cell wall through hardening that even in the absence of internal turgor allows the individual cells to stay upright against the effect of compressive forces caused by gravity (Volkmann and Baluska, 2006). Modification of cell wall composition is, therefore, a readily observed phenomenon in plants exposed to a change in *g*-force (Waldron and Brett, 1990). This type of response has been termed gravity resistance (Hoson and Soga, 2003). Experiments that are performed to study these architectural responses of plants to the effect of *g*-force are generally based on increasing its magnitude through placing the specimen into a centrifuge, or by decreasing it through exposure to omnilateral or true micro-gravity conditions (Hemmersbach et al., 1999; Hoson and Soga, 2003; **Figures 1A,B**). Omnilateral micro-gravity conditions can be produced in a clinostat or a random positioning machine by turning the specimen in rotary 2D motion or randomly in 3D, either at slow or rapid speed (Skagen and Iversen, 1999; **Figure 1C**). This does not actually alter the magnitude of the gravity force but it minimizes the effect associated with a unidirectional stimulus. True micro-gravity conditions can be achieved either on orbital platforms or during the free fall phase of sounding rocket and parabolic flights.

Plants do not only respond to a change in magnitude but also to a change in orientation of the *g*-vector. These responses are termed gravimorphogenetic and are typically expressed in form of a gravitropic behavior that is oriented in the direction of the *g*-vector (positive gravitropic) or opposed to it (negative gravitropic; Kiss, 2000; Hoson and Soga, 2003). Typical responses include the re-orientation of the root and the shoot of a plant that has been turned on its side. Numerous studies have investigated these gravitropic responses both in multicellular organisms and single-cells (Baluska and Hasenstein, 1997; Braun and Sievers, 2000; Kiss, 2000; Perbal and Driss-Ecole, 2003; Morita, 2010). Typical experiments include simple changes in the direction of the gravity

vector by reorienting the sample (**Figure 1D**). However, these experiments are frequently also conducted under micro- or hyper-gravity conditions to enhance the response and analyze dose–effect relationships. Intriguingly, in multicellular organisms, the gravitropic response typically occurs at a location that is spatially separated from the cells that are responsible for the perception of the directional signal and thus requires long-distance signaling processes about which our understanding has significantly improved in recent years (Haswell, 2003; Morita, 2010). Auxin is an important mediator of gravity response in roots and shoots (reviewed by Morita, 2010). Gravitropism in *Arabidopsis* roots is controlled by basipetally transported auxin (Rashotte et al., 2000). Consistent with the important role of the hormone, transcriptomic studies have shown that the expression of genes related to auxin biosynthesis is altered by a change in gravity level (Tamaoki et al., 2011). Several auxin transporters from the PIN and PGP families are known to be involved in the distribution and the direction of auxin fluxes. The involvement of auxin and auxin transporters in gravisensing and graviresponse emphasize the importance of investigations into the regulatory mechanisms of the action of this hormone. For a thorough and up-to-date overview of auxin signaling, we refer to recently published reviews (Zhao, 2010; Wu et al., 2011).

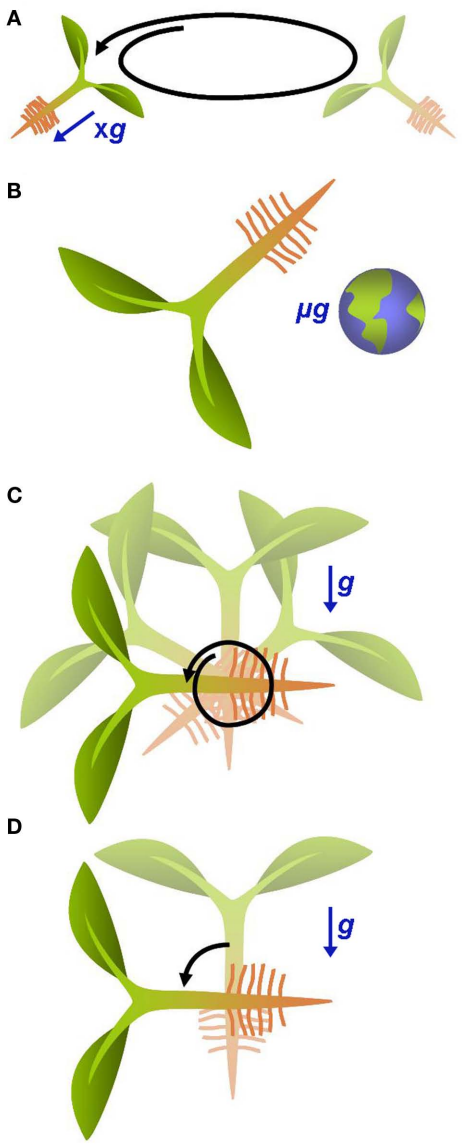


**FIGURE 1 | General principles used in gravity research.**
**(A)** Centrifugation increases the magnitude of the *g*-vector resulting in a hyper-gravity stimulus. **(B)** True micro-gravity conditions can be obtained on orbital platforms or during the free-fall phases of sounding rockets and parabolic flights. **(C)** Omnilateral micro-gravity is achieved by continuously rotating the specimen, either in 2D or 3D. This does not change the magnitude of the *g*-force, but it eliminates the effect of the unidirectional stimulus caused by the *g*-vector. **(D)** Turning a specimen changes the orientation of the *g*-vector relative to the specimen.

The challenges of cultivating plants or plant cells at micro- or hyper-*g* are manifold ranging from the complexity and spatial limitations of experimental setups in space flight conditions and centrifuges (Musgrave, 2007) to the limited time of exposure that is possible during sounding rocket (duration of 10–12 min) and parabolic flight experiments (duration of tens of seconds; Luttges, 1992). The limited duration of these experimental setups highlights the advantage of biological systems that respond within the given time frame of the respective experimental device. While intracellular signaling cascades are triggered within 1 s upon the perception of an external mechanical signal (Hejnowicz et al., 1998), metabolic cellular responses in most plants can take up to several hours or days to be measurable thus providing a critical lower time limit for the duration of experimentation (Dutcher et al., 1994; Mullen et al., 2000).

While using entire plants is necessary to study the effects on plant growth, architecture, and reproduction, studies on cellular metabolism can potentially take advantage of single-cell experimental systems. These have the advantage of being easier to observe microscopically and other experimental conditions are easier to control. In the present review we present several single-cell plant systems that have been used in the past years and that present great potential for gravity research, in particular for the investigation of the effects of gravity on plant cellular functioning and metabolism. To introduce the open questions in this field of research, it is worth summarizing how plant cells are thought to perceive the orientation and magnitude of the gravity vector. Several conceptual models have been proposed on how plant cells perceive gravity stimulation.

## CONCEPTS OF CELLULAR GRAVISENSING IN PLANTS

### STATOLITH-BASED GRAVISENSING

In the statolith-based model, the gravity signal is triggered by the movements of small bodies inside the cytoplasm that are of higher density than the surrounding cytosol – the statoliths. The cells equipped with such statoliths are called statocytes. Statoliths typically consist of starch-containing amyloplasts or crystals such as those made of barium sulfate found in *Chara* rhizoids (Sievers et al., 1996; Kuznetsov et al., 2001; Perbal and Driss-Ecole, 2003). A change in the orientation of the gravity vector relative to the orientation of the organism causes the statoliths to sediment toward the new downward facing side of the cell and their movement results

in the deformation of other sub-cellular structures (**Figure 2A**). It was thought for a long time that the moving particles exert a tensile stress on actin arrays which in turn influence the activity of membrane located mechano-sensitive ion channels (Baluska and Hasenstein, 1997; Sack, 1997; Hejnowicz et al., 1998; Morita and Tasaka, 2004). However, drug-induced disruption of the actin arrays enhances the gravity response in the roots of *Arabidopsis* and rice (Staves, 1997; Hou et al., 2004, 2003) as well as in *Arabidopsis* inflorescence stems and hypocotyls (Yamamoto and Kiss, 2002). Moreover, *Arabidopsis* mutants with reduced levels of starch-content are nevertheless able to perceive gravity signals (reviewed by Morita, 2010). Rather than the sedimenting motion it may therefore be the direct contact of amyloplasts with the endoplasmic reticulum (ER) located in the periphery of the cell that triggers the signal (Zheng and Staehelin, 2001; Perbal and Driss-Ecole, 2003; Morita, 2010). High resolution electron tomography has revealed that the force of gravity on the mass of statoliths is sufficient to locally deform the membranes of the cortical ER (Leitz et al., 2009). Clear evidence for the action of statoliths was provided by the fact that magnetophoretic displacement of statoliths in roots and shoots of higher plants as well as *Chara* rhizoids was able to induce gravitropic curvature (Kuznetsov and Hasenstein, 1996, 1997, 2001; Weise et al., 2000). These studies highlight the usefulness of micromanipulator strategies for gravitational research (Geitmann, 2006a,b, 2007). While a local membrane bending by statoliths was proposed to act in gravisensing of root columella cells (Leitz et al., 2009), it has been shown that in moss protonemata, statoliths do not need to exert pressure. The simple contact with a receptor located at the susceptible membrane (in this case the plasma membrane) suffices to elicit the response (Limbach et al., 2005). Whatever the precise biochemical signaling pathway will turn out to be, according to the statolith-based gravisensing model the cellular response depends on the intracellular motion or displacement of some sort of particle or organelle that has a higher density than the surrounding cytoplasm and that therefore sediments to the lowest region of the cell upon the re-orientation of the latter relative to the gravity vector.

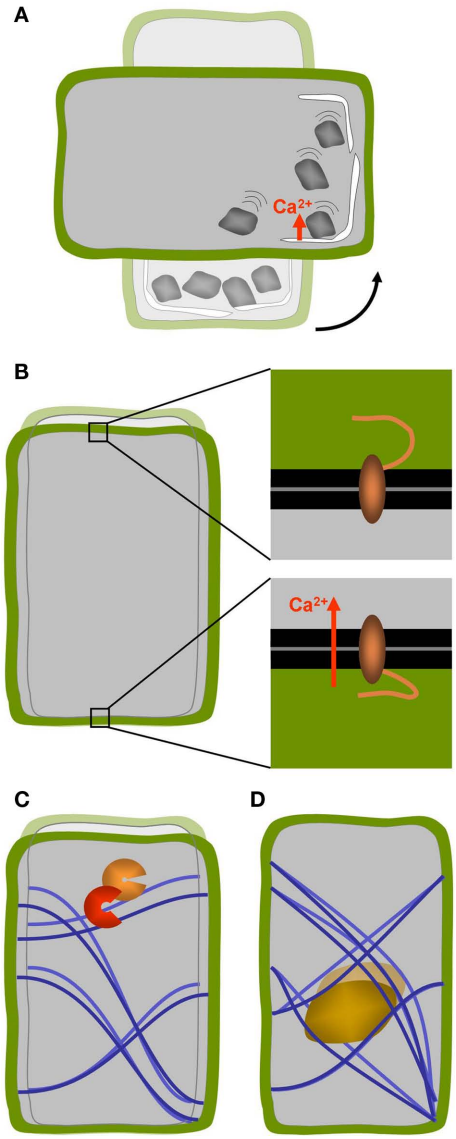


**FIGURE 2 | Concepts of cellular gravisensing in plants. (A)** In statolith-based gravisensing the sedimentation or change of position of intracellular organelles with higher density triggers a signal most likely based on a change in trans-membrane ion fluxes. **(B)** According to the gravitational pressure model the weight of the protoplast causes the forces acting on the membrane–cell wall connections at the upper and lower sides of the cells to be different. **(C)** The tensegrity model predicts that cellular distortion due to a change in *g*-force affects the pre-stress in the cytoskeletal array of the cell which in turn changes biochemical activities. **(D)** A variation of the tensegrity model is based on a change in cytoskeletal pre-stress being caused by the weight of heavy organelles that are tethered to the cytoskeletal filaments such as the nucleus.

## THE GRAVITATIONAL PRESSURE MODEL

Most plant cells are not equipped with statoliths and a second type of gravity perception mechanism needs to be in place to explain the fact that these cells nevertheless respond to changes in *g*-force. Evidence for the presence of an alternative mechanism stems from studies on mosses, fungi, and algae which show gravity-dependent growth and differentiation without the presence of statoliths (Staves, 1997). In higher plants as well, a statolith-independent pathway seems to operate. Carefully adjusted rotation of roots that maintains the statolith-equipped root cap vertical during gravitropic bending does not cause the root to abolish the bending process. This supports the view that there is a second, statocyte-independent location in the root where the gravistimulus is perceived (Wolverton et al., 2002). While at times considered a controversy, it became clear that several mechanisms of gravisensing seem to operate, possibly even in the same cell (Barlow, 1995; Sack, 1997; Hasenstein, 1999; Kiss, 2000). The presence of an alternative mechanism can explain why *Arabidopsis* mutants unable to synthesize starch display an operating gravitropic response in roots and shoots albeit diminished (Caspar et al., 1985; Hatakeda et al., 2003; Soga et al., 2005; Buizer, 2007). The gravitational pressure model provides a possible but not necessary the only explanation for this phenomenon. It suggests that the entire mass of the protoplast acts as a gravity sensor that behaves similar to a water-filled balloon that flattens when placed on a surface due to its own

weight. In this model the role of starch-filled amyloplasts would be that of increasing the overall density of the protoplast (Wayne and Staves, 1996). It is postulated that membrane proteins located at the top and bottom of cell may be activated through the action of differential tensile forces as they interact with the lower and upper cell walls, respectively (Wayne et al., 1992; Wayne and Staves, 1996; **Figure 2B**). Hitherto, the gravitational pressure model has been based on experimentation on the internodal cells of Characean algae and its relevance for graviperception in higher plants has yet to be demonstrated.

### TENSEGRITY MODEL

An alternative, but not mutually exclusive view, on how deforming forces acting on the cell as a whole could be perceived, is through the effect of cellular distortion on the mechanics or flexibility of the cytoskeletal arrays as explained by the tensegrity model of cell functioning. Although this model was developed for animal cells (Ingber, 1999), it could doubtless be transferred to plant cells. In this model, the exposure to micro-gravity decreases the internal pre-stress in the cytoskeletal array consisting of elements that resist compressive (microtubules) and tensile stresses (actin filaments). This conceptual model of cell functioning is based on the architectural principles by Buckminster Fuller (Ingber, 1993). The distortion induced change in preexisting force balance is supposed to affect local thermodynamic or kinetic parameters and thus biochemical activities (**Figure 2C**). How mechanical signals perceived at the cell surface could influence intracellular processes has been reviewed in detail (Ingber, 2006; Orr et al., 2006). However, the change in pre-stress of the cytoskeletal arrays does not necessarily need to be caused by the distortion of the outer shape of the cell but could also result from the gravity force acting on organelles attached to this network (Yang et al., 2008; **Figure 2D**). Finite element modeling has shown that the difference in density between the nucleus and the cytoplasm would be sufficient to cause a change in tensile stress that is significant enough to deform the cytoskeletal array upon application or removal of gravity (Yang et al., 2008).

While according to the tensegrity model the activities of numerous cytoplasmic enzymes are affected directly, the crucial mechanotransduction step in the statolith and pressure models is the deformation of a membrane (plasma membrane, tonoplast, mitochondria, ER) which in turn influences trans-membrane $Ca^{2+}$ fluxes and consequently the cytosolic concentration of the ion (Fasano et al., 2002; Toyota et al., 2008). Although in statocytes and gravitactic unicellular organisms, calcium was shown to be involved in graviperception as well as in signaling processes leading to a graviresponse, in non-statocyte cells in higher plants, knowledge on calcium signaling involvement in graviresponse is scant (Sinclair and Trewavas, 1997; Kordyum, 2003), and warrants further investigation.

## SINGLE-CELL SYSTEMS USEFUL FOR UNDERSTANDING STATOLITH-INDEPENDENT GRAVIPERCEPTION

Immediate and short-term responses can be difficult to assess in multicellular systems, and therefore the use of single-cells in culture has been a successful alternative approach that complements our understanding of graviperception and responses in plants. Handling of single-cells is generally easier (although typically sterile conditions have to be ensured) and depending on the parameter of interest, the response can often be observed shortly after application of the environmental trigger. Moreover, microscopy is facilitated since no neighboring cells obstruct the view, thus allowing for high spatial and temporal resolution imaging. Finally, reproducible growth conditions are more readily achieved in cell cultures since parameters such as temperature, nutrient concentrations, and pH can be tightly controlled.

Among the most intensively studied single-cell systems investigated in micro-gravity research are the protonemata and rhizoids of mosses and *Chara*, a freshwater alga. Although these cells are part of a multicellular organism, they are tip-growing individual cells which makes them readily accessible to microscopic observation. Furthermore, gravity perception and response occur in the same cell. Both protonemata and rhizoids are equipped with statoliths and important information has been gained from numerous studies on these organisms which have been exhaustively reviewed (Schwuchow et al., 1990, 1995; Sievers et al., 1996; Braun, 1997; Sack, 1997; Demkiv et al., 1999; Braun and Sievers, 2000; Braun and Wasteneys, 2000; Braun and Limbach, 2006). In the following, we will confine our overview to studies performed on single plant cell systems that are not equipped with statoliths and that offer the possibility to assess how gravity or the absence thereof affect basic plant cell functioning and metabolism.

### CELL WALL ASSEMBLY IN PROTOPLASTS

The molecular architecture of the cell wall and, by consequence, its mechanical behavior are known to be affected in many but not all plants grown under micro- and hyper-gravity conditions (Cowles et al., 1984; Waldron and Brett, 1990; Nedukha et al., 1994; Hoson et al., 1996; Nedukha, 1996; Soga et al., 1999a,b, 2001; Levine et al., 2001). Therefore, the investigation of altered *g*-force on the kinetics of the cell wall assembly process is of considerable interest. Single-cell studies to this end have been conducted on protoplasts generated from different plant systems. After the enzymatic removal of the cell wall, protoplasts generally start to regenerate a new cell wall. This is followed by cell division and formation of small cell aggregates few days later. These aggregates develop into callus tissue and, under suitable conditions, into mature plants. It is in particular the ability to regenerate mature plants from protoplast cultures that makes this experimental system interesting for applications in space exploration. Under micro-gravity conditions, cell wall formation in protoplasts isolated from *Brassica napus, Daucus carota,* and *Solanum tuberosum* is significantly delayed compared with the control samples at 1-*g* (Nedukha et al., 1994; Rasmussen et al., 1994, 1992). In particular, the content of structural components such as cellulose and hemicellulose is reduced whereas pectin is unaltered (Nedukha, 1998; Skagen and Iversen, 2000). These micro-gravity induced delays in protoplast regeneration also slow callus formation but do not prevent the process. However, under micro-*g* conditions development of intact plants is hampered, since calluses develop either roots or shoots, but not both (Iversen et al., 1999).

In these studies, peroxidase activity was measured in the regenerating protoplasts and revealed a decrease in enzyme activity compared to the ground control (Rasmussen et al., 1992). Since

peroxidase activity is involved in cell wall metabolism and cross-linking of microfibrils, this reduced activity was proposed to provide a possible explanation for the observed slow-down in cell wall regeneration. However, later experiments showed that factors other than weightlessness (such as cosmic radiation) may have contributed to or even caused this change in peroxidase activity (Skagen and Iversen, 2000).

Interestingly, another cellular feature may be the reason for retarded cell wall deposition, in particular reduced cellulose deposition: the cortical microtubule cytoskeleton. The amount of cortical microtubules at 24 h after protoplast isolation is greater in protoplasts cultured at 1-*g* than under micro-gravity (Skagen and Iversen, 2000). Moreover, while cortical microtubules assessed at 24 h after cell wall removal in recovering rapeseed protoplasts are organized in parallel arrays, they are randomly oriented in the 0-*g* sample and hence unaltered from those of the protoplast immediately after cell wall removal. Since cortical microtubules play an important role in cellulose microfibril deposition (Emons et al., 2007), the failure to reorganize may certainly influence the capacity of the cell to fully regenerate its wall, and subsequently, to divide.

## CALCIUM FLUXES IN POLAR FERN SPORES

One of the single-cell systems capable of a gravitropic response is the gametophyte of the fern *Ceratopteris richardii*. During the first 24 h of germination, the gravity vector determines the axis of development of the spore by setting an asymmetrical growth polarity creating two asymmetrical cells that will grow parallel to gravity in opposite directions (Edwards and Roux, 1998; Chatterjee and Roux, 2000). When developed in micro-gravity conditions, the spores are able to germinate but lose the spatial polarity (Roux et al., 2003). The same pattern was observed when calcium channel blockers or inhibitors such as nifedipine and eosin yellow were added to the culture medium after germination initiation (Chatterjee et al., 2000). Efflux of calcium at different cellular regions was recorded using ion selective electrodes on spores grown at 1-*g* and spores grown during parabolic flights (the *g* level fluctuated between micro-*g* and 1.8-*g*). The results revealed that the specific activation of mechano-sensitive calcium channels at the bottom of the cell is required for graviperception (Chatterjee and Roux, 2000; Salmi et al., 2011). These data confirm the notion that calcium fluxes are involved in graviperception in multicellular plants (Sinclair and Trewavas, 1997; Fasano et al., 2002; Soga et al., 2002; Hoson et al., 2010). Use of the fern spore system allowed for facilitated microscopical access and measurement of ion fluxes compared to the multicellular root cell systems. However, the mechanism by which the channels are activated and the pathways that are involved in the transduction of the gravity-dependent response are yet to be determined. Progress on the understanding of the molecular mechanism will certainly profit from advances made on other mechanoperceptive cellular systems (Orr et al., 2006; Poirier and Iglesias, 2007).

## MICROTUBULE CYTOSKELETON IN BY-2 CELLS

The microtubule cytoskeleton has multiple functions in plant cells including the guidance of the intracellular motion of organelles, the targeting of enzymes involved in cell wall assembly, chromosome separation, and cell plate formation during mitosis. When and where microtubules are assembled from tubulin and disassembled, therefore, is pivotal for cellular functioning. In weightlessness, isolated tubulin does not self-organize into parallel microtubule bands as it does in the same *in vitro* conditions on the ground (Papaseit et al., 2000). Similarly, in different types of cultured mammalian cells, the organization of the microtubule cytoskeleton is affected under real or omnilateral micro-gravity conditions (Lewis et al., 1998; Rösner et al., 2006). The finding that microtubules in regenerating protoplasts cultured under micro-gravity conditions were less organized than in the ground control provided a motivation for investigating the microtubule cytoskeleton in cultures of other single plant cell types. Similar to the protoplasts, microtubules in cultured cells dedifferentiated from tobacco hypocotyls at 1-*g* were more abundant than their counterpart grown in micro-gravity (Sato et al., 1999). The microtubules of a third single-celled system, tobacco BY-2 cells, were found to be less susceptible to a change in *g*-force, on the other hand. The BY-2 cell line was established from a callus induced on a seedling of *Nicotiana tabacum* cultivar Bright Yellow-2. Tobacco BY-2 cells grow rapidly in suspension culture and can multiply their numbers up to 100-fold within 1 week in adequate culture medium. Remarkably, exposure of BY-2 cells to micro-gravity conditions did not have any effect on cell division or cell growth. The organization of cortical microtubules was identical to that in cells cultured on Earth, and the orientation of newly deposited cellulose microfibrils was unaltered (Sieberer et al., 2009). Therefore, the tissue context does not seem to be a prerequisite for these cells to ensure cytoskeletal ordering under weightlessness. Rather it seems that the absence of a cell wall hampers microtubule organization during the initial phase of protoplast regeneration. This is consistent with the observation that microtubules in plant cells tend to align parallel to the principal direction of stress and thus respond to mechanical cues (Hush and Overall, 1991; Geitmann et al., 1997; Hamant et al., 2008). In line with this, the expression of tubulin genes is upregulated by hyper-gravity in *Arabidopsis* hypocotyls (Matsumoto et al., 2007) and cortical microtubules are reoriented from transverse to longitudinal (Soga et al., 2006). In tubulin mutants of *Arabidopsis* displaying twisted growth, hyper-gravity caused a more pronounced twisting phenotype and microtubule re-orientation was more prominent (Matsumoto et al., 2010). This led to the hypothesis that by influencing cellulose deposition, microtubules play an important role in the maintenance of a normal growth phenotype against the gravitational force (Hoson et al., 2010).

## ENDOMEMBRANE TRAFFICKING IN THE POLLEN TUBE

Experiments placed on parabolic flight and sounding rockets only make sense if the organism or cell displays a response to micro-gravity that appears within the short duration of weightlessness achieved during the flight. Assessment of both cellular growth and metabolism using these devices can therefore be exploited with cell systems in which both parameters are highly active and change rapidly upon exposure to a trigger. The fastest growing plant cell is the pollen tube, a cellular protrusion formed by the pollen grain upon contact with the receptive stigma. The pollen tube is responsible for fertilization in higher plants and hence crucial for seed and fruit formation. Pollen tubes, like protonemata, rhizoids, root hairs, fungal hyphae, and neurons, are tip-growing

cells. The growth rates of pollen tubes can be up to hundreds of micrometers per minute and sustaining this process requires the continuous deposition of cell wall material in highly controlled manner to ensure morphogenesis of a perfectly cylindrical shape (**Figure 3**).

The development of the pollen grain, or microgametophyte, occurs in the anther and comprises several, precisely regulated cell divisions (meiotic and mitotic), the assembly of a highly structured and extremely resistant cell wall, and the coordinated activation of the cytoskeleton (Honys et al., 2006; Bou Daher et al., 2011; Liu et al., 2011). Long term studies on orbital platforms have shown that pollen formation was aborted at early stages and young microspores were deformed and empty. In late developmental stages, the pollen exine was able to form but the cytoplasm seemed contracted and became disorganized (Kuang et al., 1995). However, rather than an effect of micro-gravity, these phenomena were found to be a consequence of the reduced carbon dioxide environment due to lack of convective air movement. When plants were grown in high $CO_2$ atmosphere, pollen with normal outer morphology could be obtained from *Arabidopsis* and *Brassica*. However, although the percentage of viable pollen was much higher than during the earlier experiment, fertilization did not occur (Kuang et al., 1996). Optical and electron microscopy showed that pollen grains developed in micro-gravity displayed differences in size, shape, number of mitochondria as well as an abundant presence of large starch grains absent in the pollen that developed on the ground (Kuang et al., 1995, 2005). When environmental conditions at micro-*g* such as $CO_2$, light and convection were more carefully controlled, it was possible to obtain viable embryos, seeds, and siliques in *Arabidopsis* and *Brassica* proving that pollen tube growth was indeed possible at micro-*g* (Musgrave et al., 1997; Popova et al., 2009).

At hyper-gravity the effects on pollen and fertilization vary significantly between species. While at 4-*g* seed set in *Brassica* was not affected (Musgrave et al., 2009a), the formation of siliques was significantly reduced in *Arabidopsis* (Musgrave et al., 2009b). The authors found this to be due to a reduced ability of *Arabidopsis* pollen tubes to germinate at 4-*g*. They propose that the increasing *g*-force caused the cytoplasm to exert higher than normal pressure on the cell wall disturbing the tip growth process (Musgrave et al., 2009b). The main metabolic activity of the pollen tube is the synthesis and the deposition of cell wall precursors which are indispensable for the continuing assembly of the elongating cell (Geitmann and Steer, 2006). The principal phenomenon responsible for the tube expansion is the exocytosis of vesicles containing pectins which occurs at high rates and is spatially and temporally regulated by a multitude of parameters (Chebli and Geitmann, 2007). Other cell wall components such as cellulose, xyloglucans, and callose are either deposited by exocytosis or directly synthesized at the plasma membrane. Each of these components plays

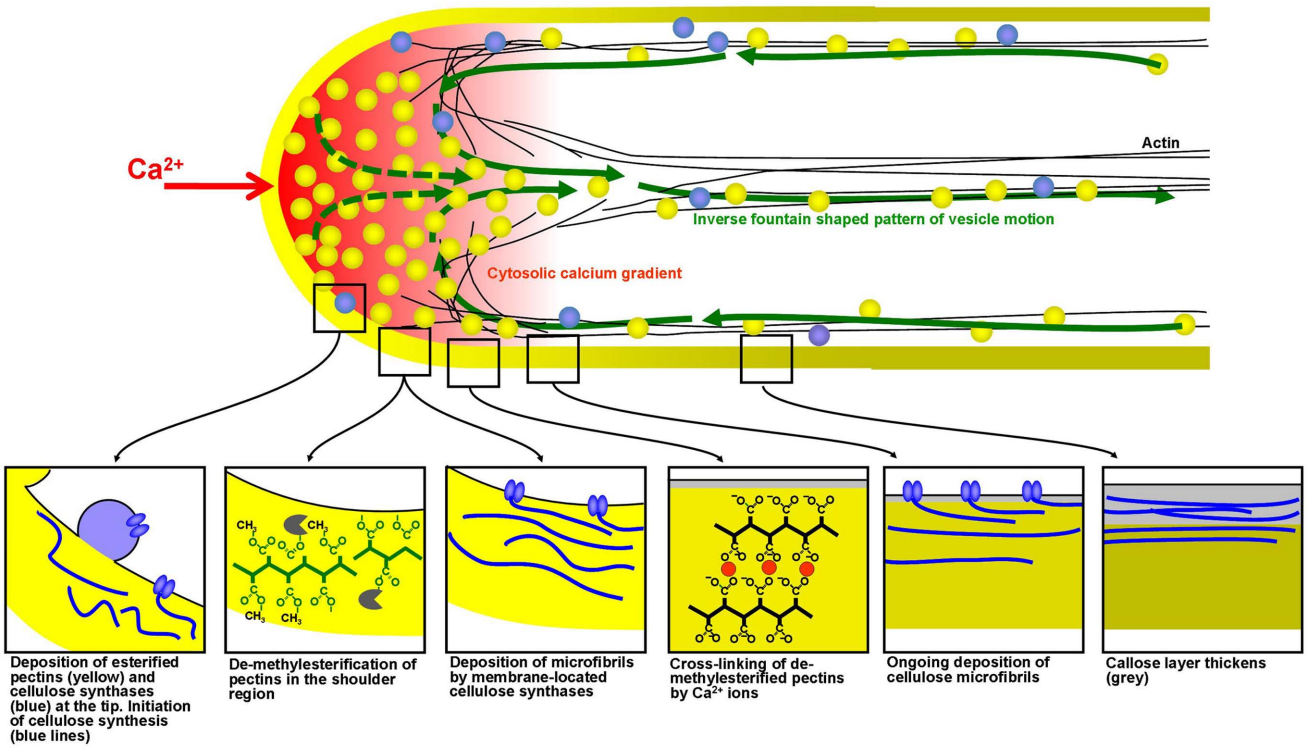


**FIGURE 3 | Spatial profile of mechanical properties and biochemical processes in the cell wall of growing pollen tubes.** Mechanical modeling has shown that the precise spatial distribution of endomembrane trafficking, cell wall assembly processes and cross-linking of cell wall polymers determines the cellular morphogenesis, and the resulting shape in growing plant cells. These processes occur particularly rapidly in pollen tubes making them an ideal model system for experiments based on short-term exposure to micro-gravity.

**Glossary**

| | |
|---|---|
| Gravimorphogenetic | Developmental change in response to the presence or the change in the orientation or magnitude of the *g*-vector |
| Graviperception | The ability of a cell to become aware of the orientation or magnitude of the gravity vector |
| Graviresponse | Physiological or morphogenetic response of a cell or a tissue to gravity induced trigger. The responding cell does not need to be identical to the perceiving cell |
| Gravisensing | Perception of gravity stimulus |
| Gravistimulus | Mechanical process caused by the presence or change in magnitude or orientation of gravity |
| Gravitropism | Ability of a plant, organ or cell to orient its growth in the direction of the gravity vector |
| Gravity resistance | Gravimorphogenetic response that serves to reinforce the organism or organ against the effect of gravity. Typically a reinforcement of the cell wall |
| Mechanotransduction | Conversion of a mechanical stimulus into a chemical intracellular signaling pathway |
| Omnilateral | From all sides |
| Statocyte | Cell specialized in graviperception and typically equipped with statoliths |
| Statolith | Intracellular body with density higher than the surrounding cytosol causing the body to sediment in the direction of the gravity vector. Either the sedimenting motion or the new position of the statolith provides the cell with information on magnitude and orientation of the gravity vector |

a defined mechanical role during pollen tube growth and in each plant species these components are distributed differently forming a characteristic spatial profile (Geitmann and Dumais, 2009; Fayant et al., 2010; **Figure 3**). Intriguingly, the pollen tube is able to compensate for the lack of one of the components by overproduction of another (Aouar et al., 2010), demonstrating that sophisticated mechanical control mechanisms must be in place to ensure that the final product is functional.

Very conveniently, pollen tubes are easily cultured *in vitro* thus allowing high resolution microscopic observations. Crucial in the present context, the high growth rate entails rapid and easily visible cellular responses upon mechanical or chemical manipulation (Geitmann and Steer, 2006; Chebli and Geitmann, 2007). The responses of *in vitro* growing pollen tubes developing under micro-*g* conditions or during clinostat rotation vary between plant species. In *Prunus persica* pollen tubes grown in a clinostat, callose plugs were four times longer than those in the control tubes and callose was spread along throughout the tube (De Micco et al., 2006a,b) indicating that cell wall assembly under altered gravity conditions is affected. Unlike somatic plant cells in which an altered cell wall assembly clearly fulfills a structural purpose since it reinforces the cell wall against the mechanical stress to which it is exposed, the performance of a pollen tube would not really be improved by a stiffer cell wall. The cellular response is therefore likely unspecific and offers the unique possibility to study the fundamental effect of altered gravity conditions on plant cell metabolism.

Cell wall assembly in the pollen tube is ensured by a high rate of intracellular trafficking targeted toward the growing end of the cell (Bove et al., 2008; Kroeger et al., 2009; Bou Daher and Geitmann, 2011). Temporal and spatial control of the vesicle fusion responsible for cell wall assembly at the growing tip is required to determine the rate and direction of growth (Fayant et al., 2010). The influx of calcium through plasma membrane located calcium channels at the tip of the cells plays an important role in the temporal and spatial regulation of the growth process (Hepler, 1997; Feijó et al., 2001; Chebli and Geitmann, 2007). Exocytosis in the tip region is accompanied by membrane endocytosis to ensure a balanced deposition rate of cell wall and membrane material. The delicate equilibrium between exocytosis and endocytosis is disturbed when *in vitro* growing pollen tubes are exposed to micro- and hyper-*g* conditions. In micro-gravity, the uptake of a fluorescent phospholipid into pollen tubes, an indication for endocytosis, is increased, whereas the contrary is true for hyper-gravity conditions (Lisboa et al., 2002). Since pollen tubes are not gravitropic (De Micco et al., 2006b), they can therefore serve as model systems that allow the investigation of statolith-independent, non-gravitropic responses of plant cells upon altered gravity conditions. Moreover, understanding the performance of this cell is crucial for future applications in space flight as successful fertilization is essential for on-board production of plant-based food.

## CONCLUSION

Experiments based on entire plants are inevitable to understand plant functioning under altered gravity conditions. Nevertheless, single-cell experimental systems have emerged as excellent tools that allow for in-depth studies of individual processes that are altered as a consequence of exposure to micro- or hyper-gravity conditions. It becomes increasingly clear that statolith mediated effects are not the only responses that contribute to plant behavior under altered gravity conditions. So far, each of the cellular systems mentioned above has been used to investigate particular aspects of cellular functioning. An important step forward will be the combined analysis of different processes on a single system to obtain a more holistic view of the involved mechanisms and to connect the dots. Secondly, identifying common principles shared by different cellular systems, as well as distinctive features that can be explained by the particular functionality of the cell wall will allow to make conclusions for general plant cell functioning. By way of example, cell wall regeneration was investigated in recovering protoplasts, but elongating tip-growing cells represent alternative systems that provides complementary information. The effect of gravity on the cytoskeleton has mostly been studied on comparably slowly developing cells such as BY-2 cells, but important information could probably gained from and short-term experimental devices could be exploited for systems in which these cellular features are highly dynamic such as growing pollen tubes. Ion fluxes, so far only studied in fern spores in the context of gravity research, could, and should equally be studied in other growing cells in which ion flux profiles have been established. Single-cell

systems allow for the use of imaging methods that operate at high spatial and temporal resolution and will therefore enable us to determine the roles of sub-cellular features such as targeted transport processes and ion fluxes that lead to a tropic response or a change in metabolic homeostasis. It will then be possible to link these results to those obtained from transcriptomic and proteomic approaches, thus making sense of the overwhelming wealth of data that these types of studies produce (Martzivanou and Hampp, 2003; Martzivanou et al., 2006; Wang et al., 2006; Babbick et al., 2007; Barjaktarovic et al., 2007, 2009). An integration of cell biological and imaging approaches with quantitative information on the expression of proteins involved in cell wall synthesis, lipid metabolism, cell division, etc., will help to validate existing models and inform future models. Crucially, a multifaceted view will guide the development of new biomechanical and structural approaches to decipher the pathways of gravisensing and graviresponse.

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